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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/766,500	01/19/2001	Craig M. Ruecker	2997-19	1098

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EXAMINER	
DAVIS, RUTH A	

ART UNIT	PAPER NUMBER
1651	16

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/766,500	RUECKER ET AL.	
	Examiner	Art Unit	
	Ruth A. Davis	1651	

-- The MAILING DATE of this communication app ars on the cover sheet with the corresp ndence address --

P riod for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 November 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19,47-56* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-19,47-56* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicant's request for continued examination has been received and entered into the case. Claim 57 has been cancelled. Claims 1 – 19 and 47 – 56 are pending and have been considered on the merits. All arguments have been fully considered.

Claim Objections

Claim 52 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim, or amend the claim to place the claim in proper dependent form, or rewrite the claim in independent form. Claim 53 recites "wherein said process is a solventless extraction process" which is also recited in the independent claim 1.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 1 – 19 and 47 – 56 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 and its dependents are drawn to a method for obtaining lipids from microorganisms, however are rendered vague and indefinite because it is unclear how a

"solventless" extraction process can include an aqueous solution, since the aqueous solution could be a solvent. Moreover the phrase "solventless extraction process" appears to contradict the process as aqueous extraction solutions and organic solvents are clearly used (as recited in claims 4 and 47 – 50, specification p.8, for example).

Claim 4 is confusing because it is unclear how adding an aqueous extraction solution is "solventless", as many aqueous extraction solutions are also solvents.

Claims 47 – 50 are indefinite because it is unclear how a "solventless" extraction process contains any amount of solvent, i.e. less than 5, 4, 2 or 1% organic solvent.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 1 – 6, 14 and 47 – 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gudin.

Applicant claims a process for obtaining lipid from microorganisms comprising lysing cells of the microorganism, treating the lysed cells with a solventless extraction process to produce a heavy layer of an aqueous solution and a light layer of lipid, separating the layers from each other and obtaining the lipid from the light layer. The microorganism is selected from algae, fungi, bacteria or protist and the step of treating the lysed cells comprises centrifuging. Specifically, the lipid is emulsified and comprises a suspension of lipid in an aqueous solution and the aqueous solution comprises solid cell material. The process further comprises adding an aqueous extraction solution to the light layer until the lipid is substantially non-emulsified. The aqueous solvent comprises less that about 5, 4, 2, or 1% organic solvent.

Gudin teaches a process which produces lipids wherein microalgae are cultured, dissolved, crushed (or lysed) and treated to produce separate layers (col.2 line 37-88). The layers are separated (col.2 line 37-55) into two phases: a lipid solution and an aqueous solution containing cellular residues (or solid cell material) wherein the treatment (or separation) is carried out via centrifuging (col.4 line 11-20). Gudin specifically teaches a phase separation, with or without using an aqueous solvent, whereby the lipid phase is separated from solid cellular residues and the aqueous phases (col.4 line 10 – 20). Gudin further teaches that the lipid phase can be further concentrated and/or purified by ultrafiltration or precipitation with ammonium sulfate (or an aqueous extraction solution) (col.4 line 24-30).

Although Gudin does not specifically teach an emulsified lipid in solution whereby it becomes substantially non emulsified, Gudin does teach a lipid solution whereby the lipid is precipitated out col.4 line 20-30). At the time of the claimed invention, it was known in the art that a lipid in solution is substantially an emulsified lipid and that by precipitating out the lipid (in this case with an aqueous extraction solution ammonium sulphate), the lipid becomes substantially non-emulsified. Furthermore, since Gudin teaches a solvent may or may not be used, it would have been well within the purview of one of ordinary skill in the art to optimize the amount of solvent as a matter of routine experimentation. Therefore, at the time of the claimed invention, one of ordinary skill in the art would have been motivated to centrifuge microorganisms via a solventless extraction process with a reasonable expectation for successfully obtaining lipids.

6. Claims 1 – 10, 12 – 19 and 47 – 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gudin in view of Barclay.

Applicant claims a process for obtaining lipid from microorganisms comprising lysing cells of the microorganism, treating the lysed cells with a solventless extraction process to produce a heavy layer of an aqueous solution and a light layer of lipid, separating the layers from each other and obtaining the lipid form the light layer. The step of treating the lysed cells comprises centrifuging. Specifically, the lipid is emulsified and comprises a suspension of lipid in an aqueous solution and the aqueous solution comprises solid cell material. The process further comprises adding an aqueous extraction solution to the light layer until the lipid is substantially non-emulsified. The microorganism is selected from algae, fungi, bacteria or

protist, specifically from the order Thraustochytriales, genus Thraustochytrium, Schizochytrium or mixtures thereof. More specifically, they are selected from microorganism with identifying characteristics of ATCC 20888, 20889, 20890, 20891, 20892, mutants thereof or combinations thereof. The microorganism is capable of growth at salinity levels of less than about 12 g/L of sodium chloride, capable of producing at least about 0.1 g/L/hour of docosahexaenoic acid (DHA) and comprises at least about 30% by weight of lipid, wherein at least about 30% of said lipid is DHA. Finally, the microorganisms are obtained from a fermentation process whereby a base selected from hydroxides, carbonated, bicarbonates or mixtures thereof is added to the fermentation broth and at least part of proteinaceous compounds are solubilized in the fermentation broth. The aqueous solvent comprises less that about 5, 4, 2, or 1% organic solvent.

Gudin teaches a process which produces lipids wherein microalgae are cultured, dissolved, crushed (or lysed) and treated to produce separate layers (col.2 line 37-88). The layers are separated (col.2 line 37-55) into two phases: a lipid solution and an aqueous solution containing cellular residues (or solid cell material) wherein the treatment (or separation) is carried out via centrifuging (col.4 line 11-20). Gudin specifically teaches a phase separation, with or without using an aqueous solvent, whereby the lipid phase is separated from solid cellular residues and the aqueous phases (col.4 line 10 – 20). Gudin further teaches that the lipid phase can be further concentrated and/or purified by ultrafiltration or precipitation with ammonium sulfate (or an aqueous extraction solution) (col.4 line 24-30).

Although Gudin does not specifically teach an emulsified lipid in solution whereby it becomes substantially non emulsified, Gudin does teach a lipid solution whereby the lipid is precipitated out col.4 line 20-30). At the time of the claimed invention, it was known in the art

that a lipid in solution is substantially an emulsified lipid and that by precipitating out the lipid (in this case with an aqueous extraction solution ammonium sulphate), the lipid becomes substantially non-emulsified. Furthermore, since Gudin teaches a solvent may or may not be used, it would have been well within the purview of one of ordinary skill in the art to optimize the amount of solvent as a matter of routine experimentation. Therefore, at the time of the claimed invention, one of ordinary skill in the art would have been motivated to centrifuge microorganisms via a solventless extraction process with a reasonable expectation for successfully obtaining lipids.

Gudin does not teach the process wherein the microalgae are from the order Thraustochytriales, genera Thraustochytrium, Schizochytrium, mixtures thereof, or microorganisms with identifying characteristics of ATCC 20888, 20889, 20890, 20891, 20892, mutants and/or combinations thereof obtained from a fermentation process. However, at the time of the invention, one of ordinary skill in the art would have been motivated to do so because Barclay teaches a process for the production of microbial products with high concentration of omega 3 highly unsaturated fatty acids, or omega-3 HUFAs, (lipids) using microorganisms or the order Thraustochytriales (abstract). Specifically, Barclay teaches the process wherein Thraustochytrium, Schizochytrium or mixtures thereof are cultured to produce high concentrations of omega-3 HUFAs (col.5 line 20-35). In addition, microorganisms with identifying characteristics of ATCC 20888, 20889, 20890, 20891, 20892 and mutants therefrom are utilized (col.5 line 45-50). Barclay teaches that such microorganisms are fermented with grain to produce the desired omega-3 HUFAs (col.8 line 50-60).

Gudin does not teach the process wherein the fermentation broth comprises solubilized proteinaceous compounds. However, at the time of the invention, one of ordinary skill in the art would have been motivated to do so because Barclay teaches that biomass comprised of proteins and carbohydrates can be recycled into the fermentor whereby it acts as a nutrient source for the Thraustochytrium (col.14 line 34-45). Although Barclay does not specifically teach solubilizing the proteins, at the time of the invention, one of ordinary skill in the art would have recognized that by mixing the proteinaceous compounds back into the fermentation broth, the material would become solubilized. Moreover, at the time of the invention, one of ordinary skill in the art would have been motivated by Barclay to solubilize proteinaceous compounds in the fermentation broth as a source of nutrients for the microorganism with a reasonable expectation of success for obtaining lipids from a microorganism.

The above references do not specifically teach the process wherein the microorganism comprises at least about 30% by weight of the lipid, are capable of producing at least about 0.1 g/L/hour of docosahexaenoic acid (DHA), wherein at least about 30% of the lipid is DHA or wherein the microorganism is capable of growth at salinity levels of less than about 12 g/L of sodium chloride. However, Barclay does teach desirable characteristics of microorganisms include high content of omega-3 HUFAs and that they are euryhaline, or able to grow in a wide range of salinity, especially a low salinity (col.6 line 42-54). In addition, Barclay names omega-3 HUFAs to include docosahexaenoic acid, of DHA (col.6 line12-38). At the time of the invention, one of ordinary skill in the art would have been motivated by Barclay to utilize a microorganism with the instantly claimed characteristics because Barclay teaches such characteristics are economically desirable for the production of omega-3 HUFAs (col.6 line 43-

47). Furthermore, at the time of the invention, one of ordinary skill in the art would have been able to recognize that optimizations of such characteristics would be desirable in a process for obtaining lipids, as demonstrated and suggested by Barclay.

The above references do not specifically teach adding a base selected from hydroxides, carbonates, bicarbonates or mixtures thereof. However, Barclay teaches that growth of the instant strains by the instant process typically becomes more alkaline during fermentation and prefer the range of pH 5.5 – 8.5 (col.9 line 34-41). At the time of the invention, one of ordinary skill in the art would have been motivated by Barclay to add a base to the fermentation broth because of the disclosed range of pH 5.5 – 8.5 that is preferred for growth. Furthermore, it would have been obvious to one of ordinary skill in the art to utilize hydroxides, carbonates, bicarbonates or mixtures thereof because they were well known bases used in the art at the time the invention was made. In support, Wagner et al. (US 4720456) teach isolation of lipids from a fermentation broth wherein the pH of the culture medium is adjusted to pH 3 – 8 by addition of alkaline compounds (or bases) (col.4 line 44-57) to include sodium hydroxide (example 9).

7. Claims 1 – 9, 11, 14 and 47 – 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gudin in view of Wagner.

Applicant claims a process for obtaining lipid from microorganisms comprising lysing cells of the microorganism, treating the lysed cells with a solventless extraction process to produce a heavy layer of an aqueous solution and a light layer of lipid, separating the layers from each other and obtaining the lipid from the light layer. The microorganism is selected from algae, fungi, bacteria or protist and the step of treating the lysed cells comprises centrifuging.

The microorganisms are obtained from a fermentation process wherein a base selected from hydroxides, carbonated, bicarbonates or mixtures thereof is added to the fermentation broth. The step of lysing said cells comprises heating the microorganisms to at least about 50C. Specifically, the lipid is emulsified and comprises a suspension of lipid in an aqueous solution and the aqueous solution comprises solid cell material. The process further comprises adding an aqueous extraction solution to the light layer until the lipid is substantially non-emulsified. The aqueous solvent comprises less that about 5, 4, 2, or 1% organic solvent.

Gudin teaches a process which produces lipids wherein microalgae are cultured, dissolved, crushed (or lysed) and treated to produce separate layers (col.2 line 37-88). The layers are separated (col.2 line 37-55) into two phases: a lipid solution and an aqueous solution containing cellular residues (or solid cell material) wherein the treatment (or separation) is carried out via centrifuging (col.4 line 11-20). Gudin specifically teaches a phase separation, with or without using an aqueous solvent, whereby the lipid phase is separated from solid cellular residues and the aqueous phases (col.4 line 10 – 20). Gudin further teaches that the lipid phase can be further concentrated and/or purified by ultrafiltration or precipitation with ammonium sulfate (or an aqueous extraction solution) (col.4 line 24-30).

Although Gudin does not specifically teach an emulsified lipid in solution whereby it becomes substantially non emulsified, Gudin does teach a lipid solution whereby the lipid is precipitated out col.4 line 20-30). At the time of the claimed invention, it was known in the art that a lipid in solution is substantially an emulsified lipid and that by precipitating out the lipid (in this case with an aqueous extraction solution ammonium sulphate), the lipid becomes substantially non-emulsified. Furthermore, since Gudin teaches a solvent may or may not be

used, it would have been well within the purview of one of ordinary skill in the art to optimize the amount of solvent as a matter of routine experimentation. Therefore, at the time of the claimed invention, one of ordinary skill in the art would have been motivated to centrifuge microorganisms via a solventless extraction process with a reasonable expectation for successfully obtaining lipids.

Gudin does not teach the process wherein the microorganisms are obtained from a fermentation broth wherein a base selected from hydroxides, carbonates, bicarbonates, or mixtures thereof are added to the broth. However, Wagner teaches a process for isolation of lipids from microorganisms obtained from a fermentation broth wherein pH of the culture medium is adjusted to pH 3 – 8 by addition of alkaline compounds (or bases) (col.4 line 44-57) to include sodium hydroxide (example 9). At the time of the invention, one of ordinary skill in the at would have been motivated to obtain the microorganisms of Gudin by fermentation with added bases because it was well known in the art to do so in methods for obtaining lipids from microorganisms, as demonstrated by Wagner. Furthermore, it would have been obvious to utilize any of the instant bases as they were well known and used bases in the art at the time the invention was made. Moreover, at the time of the invention, one of ordinary skill in the art would have been motivated by routine practice to include bases in the fermentation broth of Gudin with a reasonable expectation of success for obtaining lipids from microorganisms.

Gudin does not teach the process wherein heating the microorganism to about 50C lyses the cells. However, Wagner teaches that the growth of the cells are terminated by a temperature increase to about 60C (col.1 line 19-22). Although Wagner does not specifically teach that this temperature shock lyses the cells, at the time of the invention, one of ordinary skill in the art

would have recognized that such a step would achieve this effect. Moreover, at the time of the invention, one of ordinary skill in the art would have been motivated to heat the cells to at least about 50C with a reasonable expectation of success for terminating cell growth, or lysing the cells.

Applicant argues that the references do not teach the recited elements, specifically that the references use solvent extractions. Applicant additionally argues that Barclay adds bases at a lower amount and for enhanced growth whereas the invention requires high pH to destroy cells and solubilize proteins. Finally applicant argues that the references require additional steps not required by the claimed invention.

However, these arguments fail to persuade because Gudin specifically teaches a phase separation, with or without using an aqueous solvent, whereby the lipid phase is separated from solid cellular residues and the aqueous phases (col.4 line 10 – 20). Further, the claims do not require a high pH. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. Furthermore, while Barclay does not specifically teach solubilizing the proteins, at the time of the invention, one of ordinary skill in the art would have recognized that by mixing the proteinaceous compounds back into the fermentation broth, the material would become solubilized. Finally, while the references may require additional steps, the claims are directed to a method comprising the recited steps, indicating additional steps may be included.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ruth A. Davis whose telephone number is 703-308-6310. The examiner can normally be reached on M-H (7:00-4:30); altn. F (7:00-3:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 703-308-0196. The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4242 for regular communications and 703-308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

Ruth A. Davis; rad
January 24, 2003